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Altered Cerebrospinal Fluid Neurofilament Light Chain but Not Neurogranin Levels Are Associated with Response to Ocrelizumab Treatment in Relapsing-Remitting Multiple Sclerosis: A Preliminary Study

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Multiple sclerosis · Ocrelizumab · Neurofilament light chain · Neurogranin · Disability

Abstract

Introduction: Ocrelizumab is a CD20-targeting monoclonal antibody used for treatment of multiple sclerosis (MS). Serum and cerebrospinal fluid (CSF) neurofilament light (NFL) chain levels are reduced in MS patients under ocrelizumab treatment indicating a preventive action against neuro-axonal degeneration. Our aim, in this preliminary study, was to explore the impact of ocrelizumab treatment on synaptic integrity through assessment of neurogranin levels. ***Methods:*** Thirteen relapsing-remitting multiple sclerosis (RRMS) patients resistant to first-line immunomodulating agents were enrolled and followed up for 24 months under ocrelizumab treatment. Disease activity was monitored by periodic EDSS, MSSS, and cranial-spinal MRI assessments. No evidence of disease activity (NEDA)-3 was determined, and CSF levels of NFL (marker of neuro-axonal integrity) and neurogranin (marker of synaptic integrity) were measured by ELISA at baseline and 12-month ocrelizumab treatment. ***Results:*** Seven RRMS patients, who preserved NEDA-3 status during 24-month follow-up, showed ≥30% NFL level decrease, whereas 6 patients with stable/increased NFL levels displayed relapse, MRI lesion, or disability progression. Although most RRMS patients exhibited increased CSF levels of neurogranin under ocrelizumab treatment, patients with and without neurogranin level increase did not differ in terms of clinical features and NEDA-3 status. Baseline neurogranin levels negatively correlated with baseline EDSS scores. ***Conclusion:*** Our results confirm that NFL effectively monitors treatment response of RRMS patients under ocrelizumab treatment. Neurogranin does not appear to exhibit a similar benefit in screening of RRMS disease activity. Nevertheless, lower neurogranin levels are associated with increased disability in RRMS indicating a potential disease activity biomarker function.

Introduction

Ocrelizumab is a humanized monoclonal antibody targeting the CD20 molecule, thereby leading to a rapid depletion of B cells. The therapeutic use of this monoclonal antibody has been approved in both relapsing-remitting

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and progressive forms of multiple sclerosis (MS), an inflammatory demyelinating disease of the central nervous system. It has been speculated that immune cell subpopulations other than B cells are also positively influenced by ocrelizumab treatment and ocrelizumab may prevent neuro-axonal damage, global and regional brain atrophy, and associated cognitive decline, as well [1, 2].

Neurofilament light (NFL) chain is an abundant cytoskeletal protein exclusively expressed by neurons. Pathological processes of MS lead to an increased disruption of the neuro-axonal membrane and subsequent release of NFL into the cerebrospinal fluid (CSF) and blood. NFL is associated with MS relapsing activity and may display prognostic ability through prediction of future relapse-related disease progression and accumulation of brain damage in MRI [3]. As is the case in all immunomodulating agents, not all MS patients give favorable response to ocrelizumab treatment, prompting the utilization of biomarkers predicting the treatment response in advance. Ocrelizumab treatment significantly reduces serum and CSF NFL levels, and persistently elevated NFL level under immunomodulating drug treatment is associated with increased risk for clinical progression, disability worsening, and progression independent of relapse activity. Thus, CSF and/or serum NFL have been suggested as a feasible and reliable biomarker for screening the impact of ocrelizumab treatment on the clinical course of MS [1, 2].

Synaptic pruning is now well known to be involved in MS pathogenesis and progression of disability in MS [4, 5]. Yet there is little known about the impact of ocrelizumab on synaptic integrity and the preservation of synaptic functions. Neurogranin is a post-synaptic protein enriched in dendritic spines, is a well-established marker of synaptic integrity and viability, and its levels are influenced by neurodegeneration [6]. While neurogranin levels are well known to be increased in mild cognitive impairment and Alzheimer's disease [7, 8], neurogranin measurements have been scarcely conducted in MS patients [9, 10]. In this preliminary study, we explored the possible associations between CSF neurogranin levels and clinical features of MS and also the potential biomarker value of neurogranin in prediction and monitoring of response to ocrelizumab treatment and compared these results with those of NFL, a well-established biomarker used for screening of ocrelizumab efficacy in MS.

Methods

Subjects

In this prospective study, 13 consecutive relapsing-remitting multiple sclerosis (RRMS) patients (as per the revised McDonald criteria) [11] non-responder to interferon-beta and fingolimod treatment were enrolled. The last disease-modifying drug used for treatment of MS was fingolimod in all patients. Patients were selected on the basis of not having a relapse in the last 4 months and not having been treated with immunosuppressive or immunomodulating agents in the last 3 months. Criteria for being a non-responder were absence of a change or increase in number of attacks or having more severe attacks or displaying ≥1 contrast-enhancing lesions in cranial MRI or displaying an increase in T2 lesions identified with successive MRIs despite treatment under immunomodulating drug treatment for at least 1 year. Patients with secondary progressive MS, coexisting autoimmune diseases, cardiovascular conditions, history of malignancy, pregnancy, history of previous ocrelizumab therapy, and clinically active infections were excluded.

Enrolled patients received ocrelizumab treatment (600 mg i.v. infusion every 6 months) for 24 months. None of the patients were unable to tolerate the treatment, and there were no adverse effects associated with parenteral administration. Patients were evaluated by six-monthly follow-up visits (baseline, 6th, 12th, 18th, and 24th months) through neurological examination, contrast-enhanced cranial and spinal MRI, expanded disability status scale (EDSS), multiple sclerosis severity score (MSSS), and progression index (EDSS/disease duration) assessments. Timed 25-foot walk and nine-hole peg tests were done on the first year (12 months) of treatment. CSF samples for NFL and neurogranin level measurements were collected at baseline (1 day before commencement of ocrelizumab) and 12 months (first year) after initiating treatment. No evidence of disease activity (NEDA)-3 was determined on the basis of absence of clinical relapses, MRI evidence of disease activity, and disability worsening in the 24-month follow-up.

The study protocol was approved by the Institutional Review Board. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. All subjects provided written informed consent prior to any study-related procedure.

ELISA

CSF NFL and neurogranin levels were measured with ELISA kits (Uman Diagnostics, Umea, Sweden, and Bioassay Technology Laboratory, Shanghai, China, respectively) according to the manufacturer's instructions. Optical densities were measured at 450 nm, concentrations were calculated by reference to the standard curves, and results were expressed as pg/mL. To calculate the fold-change alterations in levels of NFL and neurogranin under 12-month ocrelizumab treatment, CSF neurogranin and NFL ratios were calculated by dividing 1st-year CSF NFL and neurogranin levels by baseline CSF NFL and neurogranin levels, respectively. Ratios over 1 indicated an increase, and ratios below 1 indicated a decrease in the levels of these markers.

Statistical Analysis

Two-group comparisons were done by Mann-Whitney U, χ^2, and paired or unpaired *t* tests, as required. Correlation studies were conducted with Pearson's correlation test. $p < 0.05$ was considered as statistically significant.

Results

Clinical Features and Follow-Up Results

Average age (± standard deviation) of the participants (9 women, 4 men) was 35.1 ± 9.9. During the first administration of ocrelizumab (baseline), average ± standard

deviation value was 10.9 ± 4.4 years for disease duration, 6.5 ± 2.1 for total attack number, 0.7 ± 0.3 for annual attack number, 4.6 ± 1.0 for EDSS, 0.5 ± 0.2 for progression index, and 5.8 ± 1.8 for MSSS. Average values for EDSS (4.7 ± 1.3), progression index (0.4 ± 0.2), and MSSS (5.4 ± 1.9) recorded at the 12-month follow-up visit were not significantly different from those of baseline values ($p = 0.466$, $p = 0.242$, and $p = 0.311$ for EDSS, progression index, and MSSS, respectively).

None of the patients discontinued ocrelizumab treatment due to inadequate treatment response or intolerance to drug administration within the first 12 months of the follow-up. However, treatment of 6 patients was switched to other disease-modulating treatments between 14 and 19 months of the follow-up due to a significant increase in disease activity (new MRI lesions in 3, EDSS increase in 4, and clinical MS relapse in 4 patients). Seven of 13 patients showed NEDA-3 status throughout the 24-month screening period.

Association between Clinical Follow-Up Features and CSF NFL/Neurogranin Levels

Baseline values for CSF NFL and neurogranin levels were 274.3 ± 101.3 pg/mL and 478.0 ± 108.2 pg/mL, respectively. Average values of NFL significantly decreased to 204.7 ± 88.4 pg/mL ($p = 0.017$), whereas neurogranin level was significantly increased to 582.5 ± 50.8 pg/mL ($p = 0.021$) on the 12th month visit (as shown in Fig. 1).

A total of 7 RRMS patients showed CSF NFL level decrease under 1-year ocrelizumab treatment and thus showed NFL ratios <1 (0.47–0.70), corresponding to 30–53% decrease in CSF NFL levels compared to baseline NFL levels. By contrast, NFL levels of the remaining 6 patients remained stable ($n = 4$) or increased ($n = 2$) (hence NFL ratio of ≥1, 1.00–1.46), corresponding to 0–46% alteration in CSF NFL levels. Seven RRMS patients showed CSF neurogranin level increase under 1-year ocrelizumab treatment and thus showed neurogranin ratios >1 (1.15–2.21), corresponding to 15–121% increase in CSF neurogranin levels. Neurogranin levels of the remaining 6 patients decreased (hence a neurogranin ratio of ≤1, 0.95–0.99), corresponding to 1–5% decrease in CSF neurogranin levels.

Since CSF samples were collected at baseline and 12th month of the study period and treatment of 6 patients was altered after the 12-month follow-up visit, the association between NFL and neurogranin levels versus clinical variables was done for the 1st year of treatment. Nevertheless, NEDA-3 was assessed for 2 years (24 months) of the study period so as to assess the longer term impact of NFL/neurogranin level alterations on the treatment efficacy of ocrelizumab.

RRMS patients with unchanged/increased and reduced NFL levels showed comparable age, sex, disease duration, total attack number, annual attack number, baseline EDSS, progression index, MSSS, and nine-hole peg score values. However, patients with reduced NFL levels showed trends toward displaying reduced EDSS, progression index, and MSSS values and improved timed 25-foot walk scores after 1-year ocrelizumab treatment (Table 1). None of these patients developed MRI lesions, MS relapse, or disability progression during the 2-year follow-up period, and thus, all 7 patients with reduced NFL levels fulfilled the criteria for NEDA-3 (as shown in Fig. 1; Table 1). CSF neurogranin levels and ratios were comparable among patients with reduced and unchanged/increased NFL levels (Table 1).

There were no significant differences among patients with unchanged/reduced and increased neurogranin levels in terms of clinical follow-up features, disability scores, and CSF NFL levels. Also, varying numbers of patients in both groups showed signs of disease progression and thus NEDA-3 status was found in comparable number of RRMS patients with or without CSF neurogranin alteration (as shown in Fig. 1; Table 2). Congruently, NEDA-3 patients showed significantly reduced CSF NFL level and ratio values than EDA-3 patients under ocrelizumab treatment, whereas CSF neurogranin levels and ratios were identical among NEDA-3 and EDA-3 patients (Table 3).

Correlation analysis showed significant correlation between baseline CSF NFL levels and 25-foot walk test ($p = 0.026$; $R = 0.635$), indicating that RRMS patients with higher CSF NFL levels tend to need longer time to complete the 25-foot walk. Another significant correlation was between baseline CSF neurogranin level and baseline EDSS score ($p = 0.021$; $R = -0.629$), indicating that patients with lower CSF neurogranin levels tended to have increased EDSS scores and disability. No significant correlation could be found between baseline/1st-year NFL/neurogranin levels versus other variables of the study and between NFL levels versus neurogranin levels.

Discussion

In this study, a decrease of at least 30% in CSF NFL levels was associated with NEDA-3 under 24 months of ocrelizumab treatment. Thus, our results confirm previous studies having shown decreased serum and/or CSF NFL levels in MS patients with stable disease activity under ocrelizumab treatment [1, 2]. As shown in Figure 1, RRMS patients with higher baseline NFL levels showed NEDA-3 as long as they showed a notable decrease in NFL levels in the first-year follow-up, whereas those with lower but unchanging or increasing NFL levels exhibited signs of clinical disease

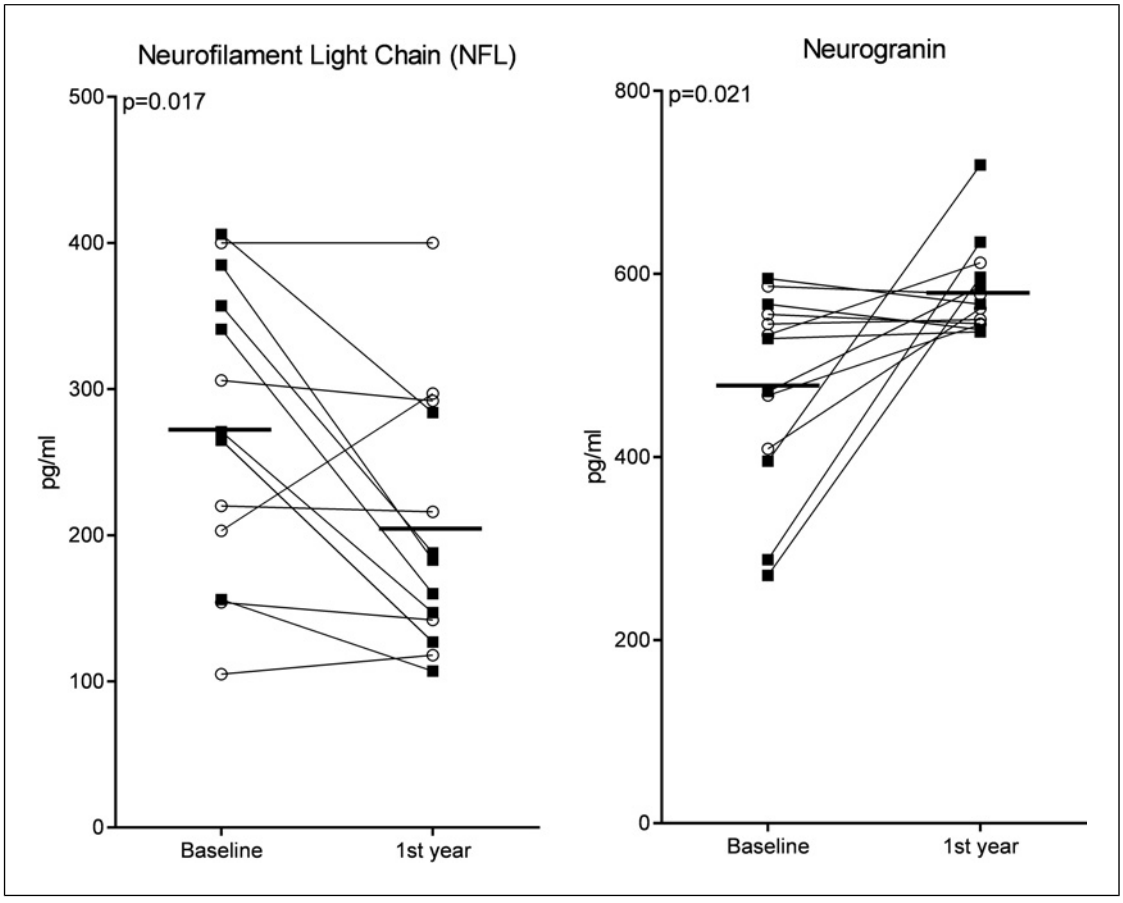

Fig. 1. CSF NFL chain and neurogranin levels of RRMS patients before (baseline) and 1 year (12 months) after ocrelizumab treatment. Full squares represent patients with NEDA-3 in the 2-year follow-up period after commencement of ocrelizumab. Thick horizontal lines indicate mean values. Statistical analysis was done by paired Student's *t* test, and *p* values were shown on the upper left corner of the panels.

activity. Thus, these results also confirm that it is the persistence of NFL levels in the follow-up period, rather than baseline NFL values that is associated with increased disease activity under immunomodulating drug treatment [1].

Notably, a significant proportion of our RRMS patients showed EDA-3 status under ocrelizumab treatment and required a drug switch. This can be explained by the fact that, as per the national regulations regarding the use of ocrelizumab in our region, all enrolled patients were unresponsive to two different immunomodulating medications and, with an average EDSS of 4.6, some enrolled participants represented MS cases that were candidates to entering a transitional stage from the relapsing to progressive form of MS.

While synaptic pruning has been implied to be involved in MS pathogenesis [4, 5, 12, 13], markers of synaptic disintegration have been scarcely investigated in MS. Although neurogranin levels show increased baseline values and predict cognitive decline in patients with mild cognitive impairment, Alzheimer disease and other disorders interfering with cognitive functions [6–8], in previous studies CSF neurogranin levels of MS patients, have been shown to be lower than those of healthy controls [9, 10]. A putative explanation could be that synaptic pruning is an actively ongoing process in primary neurodegenerative disorders, thus leading to continuously increased synapse breakdown product levels in the CSF, whereas, in MS, synaptic disintegration is a more insidious and slow-paced process that can be prevented by immunomodulating drugs. Additionally, production turnover of synaptic proteins might also have been reduced in RRMS patients because of neuroinflammation leading to reduced neurogranin levels. Our patients with reduced CSF neurogranin levels showed increased EDSS scores emphasizing once again the involvement of synaptic dysfunction in disability accumulation in MS [4].

Table 1. Comparison of clinical features and neurogranin levels of RRMS patients showing unchanged/increased and reduced CSF NFL levels under ocrelizumab treatment

	RRMS with unchanged/ increased NFL (n = 6)	RRMS with reduced NFL (n = 7)	p value
Age, years	38.7±9.9	32.0±9.5	0.121
Sex (women/men)	4/2	5/2	>0.999
Disease duration, years	9.2±1.9	12.4±5.5	0.191
Total attack number	5.8±1.8	7.1±2.3	0.141
Annual attack number	0.7±0.3	0.7±0.4	0.448
EDSS (baseline)	4.8±0.7	4.4±1.3	0.242
Progression index (baseline)	0.5±0.1	0.4±0.3	0.220
MSSS (baseline)	6.6±0.7	5.1±2.2	0.068
EDSS (1st year)	5.3±0.7	4.1±1.5	0.054
Progression index (1st year)	0.5±0.1	0.4±0.2	**0.045**
MSSS (1st year)	6.5±0.6	4.6±2.2	**0.033**
Nine-hole peg test (right hand; 1st year)	45.3±37.2	26.6±6.6	0.137
Nine-hole peg test (left hand; 1st year)	36.7±6.2	38.1±24.5	0.441
Timed 25-foot walk (1st year)	16.8±4.3	9.1±3.0	**0.003**
New MRI lesion on 2-year follow-up	3 cases	None	**0.033**
Disability progression on 2-year follow-up	4 cases	None	**0.009**
Clinical relapse on 2-year follow-up	4 cases	None	**0.009**
Patients with NEDA-3	None	7 cases	**0.0003**
CSF neurogranin level, pg/mL (baseline)	516.2±65.7	445.3±130.9	0.119
CSF neurogranin level, pg/mL (1st year)	565.5±26.0	597.1±63.7	0.131
CSF neurogranin ratio*	1.1±0.2	1.5±0.6	0.072

Parametric variables are depicted as mean ± standard deviation. All continuous parametric variables were compared by unpaired Student's t test, categorical variables were compared by χ^2 test, and disability scores were compared by Mann-Whitney U test. Significant p values are denoted by bold characters. EDSS, expanded disability status scale; MSSS, multiple sclerosis severity score; NEDA-3, no evidence of disease activity (absence of clinical relapses, MRI evidence of disease activity and disability worsening) in 2-year follow-up. *CSF neurogranin ratio is calculated by dividing 1st-year CSF neurogranin level by baseline CSF neurogranin level.

Also of note, in previous studies, CSF neurogranin levels were not altered by fingolimod, interferon-beta, and natalizumab treatments [9, 10]. By contrast, in our study, ocrelizumab treatment showed trends toward enhancing CSF neurogranin levels, implying that this monoclonal antibody might have some impact on synaptic integrity. Nevertheless, no significant association could be established between neurogranin levels and any of the clinical outcome measures arguing against a biomarker role for this molecule in monitoring of ocrelizumab treatment. However, the significant correlation between baseline neurogranin and EDSS scores suggests that neurogranin might potentially be used as a biomarker of disease activity in MS. As previously shown by others [10], neurogranin levels are not correlated with markers of neuro-axonal degeneration and glial activation. Also in our study, CSF NFL and neurogranin levels did not correlate and patients with reduced and increased NFL under ocrelizumab treatment showed identical neurogranin levels. These results indicate that synaptic disintegration contributes to MS pathology through divergent tracks that are separate from those used by factors of neurodegeneration and neuroinflammation.

Table 2. Comparison of clinical features and NFL levels of RRMS patients showing unchanged/increased and reduced CSF neurogranin levels under ocrelizumab treatment

	RRMS with unchanged/ reduced neurogranin (n = 6)	RRMS with increased neurogranin (n = 7)	p value
Age, years	32.5±6.0	37.3±12.3	0.194
Sex (women/men)	3/3	6/1	0.164
Disease duration, years	9.8±3.2	11.9±5.3	0.209
Total attack number	7.3±2.3	5.9±2.0	0.119
Annual attack number	0.8±0.3	0.6±0.3	0.088
EDSS (baseline)	4.4±0.7	4.8±1.1	0.137
Progression index (baseline)	0.5±0.2	0.5±0.2	0.368
MSSS (baseline)	5.7±1.6	5.9±2.0	0.419
EDSS (1st year)	4.2±1.3	5.1±1.2	0.109
Progression index (1st year)	0.4±0.2	0.4±0.2	0.461
MSSS (1st year)	5.2±2.0	5.6±1.9	0.355
Nine-hole peg test (right hand; 1st year)	27.8±7.1	31.5±5.4	0.202
Nine-hole peg test (left hand; 1st year)	39.8±25.4	35.4±9.3	0.350
Timed 25-foot walk (1st year)	13.0±7.3	12.9±3.3	0.478
New MRI lesion on 2-year follow-up	2 cases	1 case	0.416
Disability progression on 2-year follow-up	2 cases	2 cases	0.853
Clinical relapse on 2-year follow-up	3 cases	1 case	0.164
Patients with NEDA-3	3 cases	4 cases	0.797
CSF NFL level, pg/mL (baseline)	244.9±99.1	299.6±103.6	0.176
CSF NFL level, pg/mL (1st year)	198.3±79.4	201.2±101.5	0.408
CSF NFL ratio*	0.9±0.4	0.7±0.2	0.194

Parametric variables are depicted as mean ± standard deviation. All continuous parametric variables were compared by unpaired Student's t test, categorical variables were compared by χ^2 test, and disability scores were compared by Mann-Whitney U test. EDSS, expanded disability status scale; MSSS, multiple sclerosis severity score; NEDA-3, no evidence of disease activity (absence of clinical relapses, MRI evidence of disease activity and disability worsening) in 2-year follow-up. *CSF NFL ratio is calculated by dividing 1st-year CSF NFL level by baseline CSF NFL level.

Table 3. Comparison of CSF NFL chain and neurogranin levels of RRMS patients with and without "NEDA-3" under ocrelizumab treatment

	EDA-3 (n = 6)	NEDA-3 (n = 7)	p value
CSF NFL level, pg/mL (baseline)	231.1±106.7	311.4±87.0	0.116
CSF NFL level, pg/mL (1st year)	244.2±106.2	170.8±57.7	**0.044**
CSF NFL ratio*	1.1±0.2	0.6±0.1	**0.0003**
CSF neurogranin level, pg/mL (baseline)	516.2±65.7	445.3±130.9	0.119
CSF neurogranin level, pg/mL (1st year)	565.6±26.0	597.1±63.7	0.131
CSF neurogranin ratio*	1.1±0.2	1.5±0.6	0.072

All values are shown as mean ± standard deviation. Statistical comparisons were done by unpaired Student's t test. Significant p values are denoted by bold characters. *CSF neurogranin and NFL ratios are calculated by dividing 1st-year CSF NFL or neurogranin level by baseline CSF NFL or neurogranin level.

A limitation of our study was the low case number due to its preliminary and explorative nature. Second, due to the lack of advanced technology for measurement of serum NFL levels in our facility, NFL assessment was done by CSF measurements and this prevented procurement of more than two samples that would enable more effective evaluation of NFL production kinetics. Third, all of our outcome measures were restricted with somatic neurological aspects of MS. In future studies, we recommend the assessment of impact of neurogranin level alterations on the cognitive outcome measures, as well, since synaptic loss might have a central significance for the cognitive decline encountered in MS patients [13]. Finally, we would recommend NFL and neurogranin measurements to be done with other drugs used in MS therapy.

Conclusion

In brief, our results suggest that neurogranin may not have the same biomarker value as NFL in screening of the ocrelizumab treatment response. Neurogranin (and putatively other synaptic disintegration markers) and neuro-axonal degeneration markers appear to be separately involved in MS pathogenesis, and ocrelizumab putatively shows trends toward restoring synapse functions. Nevertheless, due to its association with the EDSS score, neurogranin measurement might potentially be used to monitor disease activity in MS.

Statement of Ethics

All the participants provided written informed consent. The study protocol was approved by the Ethical Committee of Istanbul University (Reference Number: 812536). All subjects provided written informed consent prior to study-related procedure.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Conceptualization, supervision, and project administration: E.T., R.T., and V.Y.; methodology: E.A, V.Y., and T.K.; software: D.Ö.Y.; validation, formal analysis, investigation, and visualization: T.K, R.E., and A.S.D.; resources: E.A. and D.Ö.Y.; data curation: T.K., E.A., and D.Ö.Y; writing – original draft preparation: T.K., R.T., and E.T.; writing – review and editing: V.Y. and E.A.; and funding acquisition: E.A. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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